AMRL-TR-67-175

DISTRIBUTION AND EXCRETION OF ^{14}C-MONOMETHYLHYDRAZINE

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NOVEMBER 1967

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89–544, "Laboratory Animal Welfare Act," August 24, 1967.

700 - March 1968 - CO455 - 26-566

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FOREWORD

This study was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry." The work was performed from September 1965 to September 1966 in the Toxicology Branch, Toxic Hazards Division, Biomedical Laboratory. Valuable assistance rendered by Major Ralph Ziegler, Major Vernon Carter, Captain Gale Taylor and Miss Marilyn George is gratefully acknowledged.

The material presented in this Technical Documentary Report was presented at the Society of Toxicology Sixth Annual Meeting on 25 March 1967 in Atlanta, Georgia.

This technical report has been reviewed and is approved.

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ABSTRACT

A total of 20 mice, 20 rats, 17 dogs, and 16 monkeys received intraperitoneal injections of ^{14}C-monomethylhydrazine (MMH) at doses of 22 mg/kg (mice), 15 mg/kg (rats), and 10 mg/kg (monkeys and dogs). At 2, 4, 8, and 24 hours after exposure, representative samples of approximately 20 tissues from each animal were processed for ^{14}C assay using liquid scintillation counting techniques. Both blood and urine samples were simultaneously analyzed by a chemical colorimetric method for unchanged MMH, and the results were correlated with total ^{14}C content. Results of the ^{14}C assays indicated that the mouse, rat, and monkey excreted twice as much as the dog in the first 2 hours, and that all 4 species excreted 25-40% of the total dose by 24 hours after injection. Approximately 50% of the total ^{14}C excretion, at all experimental times, was apparently unchanged MMH as implied by the colorimetric results. Tissue distribution of ^{14}C showed the highest concentrations in liver, kidney, bladder, pancreas, and blood serum. Both clinically and pathologically, the dog was apparently much more susceptible than the other species tested to the toxic effects of MMH and to severe kidney damage.

SECTION I

INTRODUCTION

Monomethylhydrazine is a fuel component of missile and rocket propulsion systems. While the intoxicating effects of the hydrazines as a generically similar family have been known and studied for many years, it has become increasingly apparent that they do not all have the same mode of toxic action. They are all convulsants, however, and the methyl-derivatives appeared to have certain other properties in common. When the therapeutic effectiveness of pyridoxine, one of the B-6 analogues, against the toxic effects of unsymmetrical dimethylhydrazine was discovered, we assumed that this agent would be equally effective against monomethylhydrazine. Should the results of experiments with pyridoxine and monomethylhydrazine have been unequivocal, from an applied research standpoint we could have had some confidence in the efficacy of therapy and could have concentrated our efforts on areas other than the basic mechanisms of toxic action.

From our own work and the work of others, however, the reports on the efficacy of pyridoxine for monomethylhydrazine intoxication did not unequivocally agree. The various workers reporting had used different species of test animals in many instances, and since we were going to investigate the metabolic fate of this material, we decided to use radioactive tracer techniques and as many different species of animals as possible to save time and to have comparative data using the same stock material. Our study, therefore, was designed to utilize rats, mice, monkeys, and dogs and to cover a period of 8 months.

SECTION II

METHODS

^{14}C-Monomethylhydrazine (MMH) was synthesized by a commercial source[1] and was provided with a specific activity of 3.1 uc/mM (60 uc/ml). The material is water soluble, and aqueous dilutions were prepared fresh immediately prior to use.

Experimental Animals

The total number of test animals used in these experiments was as follows: 20 Sprague-Dawley rats weighing 184 to 384 grams; 20 Swiss-

[1]Research Chemicals, Orlando, Florida

strain mice weighing 27 to 47 grams; 17 mongrel dogs weighing 6.9 to 14.4 kilograms; and 16 Macaca mulatta monkeys weighing 2.2 to 3.5 kilograms. Dogs and monkeys were equally distributed as to sex.

Animal Procedures

All animals were fasted overnight and weighed just prior to use. The test material (^{14}C-MMH) was administered by intraperitoneal route, and doses were varied according to species based on preliminary dose response studies and other pertinent data obtained both in our own laboratories and by Dost et al (1966). Mice received ^{14}C-MMH at a dose of 22 mg/kg; dogs and monkeys, 10 mg/kg; rats, 15 mg/kg and 22 mg/kg. Due to the unexpectedly severe toxic response of rats to 22 mg/kg after 6 hours, the dose in rats was reduced to 15 mg/kg for the 8- and 24-hour experimental post-injection holding periods. Groups of 4 or 5 animals each were injected with ^{14}C-MMH and maintained in individual metabolism cages (or chairs, in the case of monkeys) for 2, 4, 8, or 24 hours. Excreted urine was collected for each experimental period, and bladder urine was obtained at necropsy. Each animal was processed individually, except for the mice which were pooled in groups of 5 to obtain sufficient tissue material for analysis.

All animals were killed by pentobarbital sodium anesthesia and exsanguination. Blood samples were collected at this time. Necropsies were performed immediately, and whole organs were removed, weighed, and processed for liquid scintillation ^{14}C counting techniques as described by Back et al (1963). Digestion of tissue samples, 100 to 150 mg, in 3 ml Hydroxide of Hyamine-10X [R] was accomplished by overnight maintenance in a 56 C waterbath.

Clinical observations of toxic effects such as emesis or convulsions were duly noted and recorded.

Analytical Procedures

Blood serum samples were analyzed for MMH by the colorimetric procedure described by Reynolds and Thomas (1965).

Urine volumes were recorded and samples were likewise analyzed by a slight modification of the same technique. This modification consisted of using a 10-tube dilution of urine in order to circumvent interfering components which inhibited the color reaction of MMH with the p-dimethyl-aminobenzaldehyde reagent. The only other deviation from the original procedure involved extraction of the final color complex into ethyl

[R] Trademark of Rohm and Haas

acetate as recommended by Reynolds[1] to insure absolute lack of turbidity. The urine dilution technique was previously validated by in vitro recovery studies which indicates 98 to 100% accuracy.

Efforts to utilize the preceding method or any modification thereof to analyze tissue samples for MMH content were unsuccessful. Therefore, distribution results for MMH in tissues are given solely in terms of ^{14}C radioactivity.

Additional serially collected blood samples from some dogs and monkeys were also analyzed for methemoglobin by the method of Hainline (1965).

SECTION III

RESULTS

More than 20 tissues from each animal were analyzed for ^{14}C activity, and results were calculated in terms of monomethylhydrazine.

Since only the dog and monkey are comparable to each other (because of the differences in doses among the various species), and since the rats and mice received the same dose only for the 2- and 4-hour study, it is of interest that the same four tissues had the highest concentrations of ^{14}C, in all four species. These tissues were blood serum, liver, kidneys and bladder. Tables I thru IV show the values obtained in micrograms per gram of tissue for all four time periods. Note particularly the difference in time to peak concentration between the monkey and the dog. In every tissue analyzed, the dog showed the highest values at 4 hours after injection, while the monkey, just as consistently, showed its highest values at 2 hours after exposure. The mouse more closely resembled the dog in this respect, while the rat was unlike any of the others in that there was no apparent consistent pattern relative to time.

This same pattern is demonstrated in tables V thru VIII which depict the recovery in all body tissues examined, in terms of percent of dose administered. There are appreciable amounts still present in the tissues at 24 hours after exposure.

Table IX shows the radioactivity present in urine samples at the various time periods, again expressed as micrograms per milliliter in terms of monomethylhydrazine. Note again the high concentration in monkey urine at 2 hours and in dog urine at 4 hours.

[1]Personal Communication

TABLE I

DISTRIBUTION OF ^{14}C-MMH IN DOGS (ug/g)[a]

TISSUES	HOURS AFTER DOSE[b]			
	2	4	8	24
Blood Serum	7.8 (5.6-9.9)	8.1 (5.1-11.8)	4.0 (2.1-7.1)	3.0 (1.8-4.1)
Spleen	3.1 (0-6.7)	3.7 (0-7.9)	*	*
Lungs	2.5 (0-5.3)	3.7 (0-6.3)	1.0 (0-3.9)	0.9 (0-2.1)
Heart	2.4 (0-4.0)	3.1 (0-5.0)	1.5 (0-4.1)	0.8 (0-2.1)
Liver	12.7 (5.7-18.6)	16.6 (0-35.5)	12.5 (6.8-21.6)	6.3 (3.3-9.4)
Kidneys	9.8 (5.4-13.7)	14.3 (10.0-17.7)	8.0 (5.4-11.3)	1.9 (0-2.9)
Intestine	5.6 (3.6-6.3)	7.2 (3.3-10.9)	4.8 (3.4-7.9)	3.6 (1.4-5.5)
Muscle	2.2 (0-3.8)	3.1 (0-6.4)	1.0 (0-3.6)	*
Brain				
Medulla	2.5 (0-3.3)	3.2 (1.0-4.5)	2.1 (1.2-3.5)	0.8 (0-1.3)
Cerebellum	4.0 (2.0-5.2)	4.3 (1.2-6.4)	3.1 (2.2-4.7)	1.0 (0-2.0)
Cerebrum	4.0 (2.3-4.7)	5.5 (2.4-7.3)	3.2 (1.6-5.4)	1.2 (0-2.1)
Mid-brain	3.5 (1.4-4.6)	4.0 (1.4-5.4)	2.7 (1.6-4.0)	0.7 (0-1.8)
Bladder	8.1 (4.5-13.5)	9.7 (1.5-16.0)	5.9 (1.8-12.8)	2.6 (1.6-3.0)
Testes	6.1 (5.5-6.7)	4.6 (2.1-7.0)	2.6 (2.2-3.0)	0.7 (0-1.8)
Ovaries	3.5 (1.9-5.1)	4.8 (3.8-5.8)	3.5 (3.3-3.7)	2.1 (0-2.1)
Thyroid	2.5 (0-5.8)	5.1 (0-10.5)	1.6 (0-4.3)	0.8 (0-2.0)
Pancreas	6.4 (2.9-8.4)	8.5 (3.7-10.6)	5.7 (2.6-9.1)	2.5 (1.1-4.8)
Adrenals	4.6 (2.1-6.0)	6.9 (0-11.4)	4.6 (2.8-8.4)	2.7 (0-4.2)
Pituitary	3.9 (0-5.8)	2.6 (0-7.1)	2.2 (0-3.0)	0.9 (0-1.9)
Sp. Fluid	5.9 (0-10.4)	4.7 (2.9-6.1)	3.0 (0-4.2)	0.8 (0.5-1.1)
Urine	341 (151-731)	802 (274-1646)	507 (15-993)	109 (52-137)

[a]Values expressed as mean and range derived from at least 4 dogs per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 10 mg/kg

*Not detectable

TABLE II

DISTRIBUTION OF ^{14}C-MMH IN MONKEYS (ug/g)[a]

TISSUES	HOURS AFTER DOSE[b]			
	2	4	8	24
Blood Serum	9.2 (5.0-12.7)	6.2 (4.5-7.5)	4.3 (3.6-5.0)	1.0 (0-1.7)
Spleen	5.5 (3.8-6.7)	0.3 (0-1.2)	1.1 (0-4.2)	*
Lungs	6.0 (5.4-6.4)	1.6 (0-2.9)	1.6 (0-2.5)	*
Heart	4.5 (3.1-5.8)	2.3 (1.3-3.3)	1.1 (0.7-1.4)	*
Liver	11.7 (9.0-13.5)	7.7 (3.5-10.9)	7.1 (5.2-9.3)	1.4 (0-3.6)
Kidneys	16.3 (14.6-18.4)	8.2 (5.1-13.1)	6.1 (2.5-11.2)	0.6 (0-2.2)
Intestine	6.5 (5.4-7.9)	3.5 (2.6-4.6)	3.0 (2.0-3.6)	2.1 (1.1-2.8)
Muscle	3.5 (2.6-3.9)	1.0 (0-2.1)	0.8 (0-2.2)	*
Brain				
Medulla	3.7 (2.3-4.8)	2.4 (1.1-3.5)	1.9 (1.3-2.2)	0.3 (0-0.7)
Cerebellum	5.0 (3.6-6.4)	3.1 (1.7-3.5)	2.0 (1.6-2.3)	0.3 (0-0.7)
Cerebrum	4.8 (4.0-5.5)	2.6 (1.7-3.5)	2.5 (1.8-3.0)	0.4 (0-0.9)
Mid-brain	4.3 (3.4-5.3)	2.7 (1.2-4.5)	1.9 (1.4-2.5)	0.2 (0-0.7)
Bladder	15.9 (4.7-30.0)	4.9 (2.9-6.9)	4.1 (1.6-7.5)	1.2 (0-2.5)
Testes	7.0 (6.8-7.2)	3.3 (2.9-4.4)	1.4 (0.6-2.1)	0.4 (0-0.8)
Ovaries	4.5 (4.3-4.7)	3.4 (2.6-5.4)	2.4 (2.3-2.6)	0.8 (0-1.5)
Thyroid	1.6 (0-3.7)	1.2 (0-2.6)	0.3 (0-1.3)	0.9 (0-3.4)
Pancreas	5.6 (4.2-6.2)	4.0 (1.0-6.3)	3.5 (0.9-6.5)	1.3 (0-1.9)
Adrenals	4.5 (3.0-5.5)	1.4 (0-2.4)	3.4 (0.7-6.5)	0.4 (0-1.7)
Pituitary	7.5 (4.2-9.6)	3.3 (2.1-4.0)	1.5 (0-3.0)	*
Sp. Fluid	7.2 (4.3-9.6)	3.8 (2.4-5.3)	4.6 (2.9-7.1)	1.1 (0.9-1.3)
Urine	885 (660-1196)	341 (140-345)	290 (205-334)	233 (170-612)

[a]Values expressed as mean and range derived from at least 4 monkeys per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 10 mg/kg

*Not detectable

TABLE III

DISTRIBUTION OF ^{14}C-MMH IN RATS (ug/g)[a]

ORGAN TISSUES	HOURS AFTER DOSE			
	2[b]	4[b]	8[c]	24[c]
Blood Serum	8.0 (5.7-11.6)	11.6 (6.5-22.6)	5.0 (3.5-6.1)	0.7 (0.3-1.0)
Spleen	3.1 (0-7.8)	*	*	*
Lungs	2.6 (0-5.4)	2.6 (0.8-5.2)	2.7 (0-3.0)	*
Heart	2.1 (0-4.1)	2.4 (0-2.4)	*	*
Liver	5.2 (0-10.3)	10.4 (3.5-17.7)	2.7 (1.4-3.5)	*
Kidneys	15.6 (5.7-33.8)	9.5 (3.2-17.3)	3.2 (1.8-2.9)	*
Intestine	5.3 (5.0-6.8)	6.9 (5.0-8.3)	5.8 (3.8-8.9)	1.0 (0.9-1.2)
Muscle	2.1 (0-3.8)	3.2 (1.1-6.9)	1.3 (0.5-1.5)	*
Brain	4.8 (4.3-6.0)	4.6 (3.4-6.3)	1.2 (0-1.6)	*
Bladder	9.6 (5.4-12.5)	6.8 (1.6-14.0)	7.0 (4.1-7.8)	1.3 (1.0-1.8)
Testes	Not Done	5.7 (5.3-5.9)	2.4 (2.1-2.9)	*
Urine	170 (72-200)	1472 (569-2183)	571 (338-747)	240 (183-342)

[a]Values expressed as mean and range derived from at least 5 rats per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 22 mg/kg

[c]Injected dose = 15 mg/kg

*Not detectable

TABLE IV

DISTRIBUTION OF ^{14}C-MMH IN MICE (ug/g)[a]

ORGAN TISSUES	HOURS AFTER DOSE[b]		
	2	4	24
Blood Serum	2.9	4.2	2.7
Spleen	*	5.6	3.4
Lungs	*	3.3	2.3
Heart	*	2.1	1.7
Liver	4.1	7.9	6.6
Kidneys	0.9	7.1	4.4
Intestine	2.2	6.1	3.9
Muscle	*	1.2	0.8
Brain	1.0	2.8	1.0
Bladder	7.3	10.5	3.9
Testes	7.3	3.1	1.8
Urine	548	469	141

[a]Values derived from pooled tissues and fluid samples from 5 mice per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 22 mg/kg at all times

*Not detectable

TABLE V

PERCENT OF INJECTED DOSE RECOVERED IN DOGS[a]

TISSUES	HOURS AFTER DOSE[b]			
	2	4	8	24
Blood Serum	4.90	4.20	2.20	1.60
Spleen	0.07	0.10	*	*
Lungs	0.20	0.30	0.05	0.05
Heart	0.20	0.20	0.09	0.05
Liver	3.40	4.70	2.97	1.55
Kidneys	0.50	0.60	0.35	0.08
Intestine	2.60	3.30	2.20	1.50
Muscle	10.10	14.10	4.45	*
Brain	0.30	0.30	0.17	0.05
Bladder	0.10	0.06	0.03	0.01
Testes and Ovaries	0.05	0.03	0.01	<0.01
Thyroid	<0.01	<0.01	<0.01	<0.01
Pancreas	0.10	0.10	0.09	0.05
Adrenals	<0.01	<0.01	<0.01	<0.01
Pituitary	<0.01	<0.01	<0.01	<0.01
Sp. Fluid	0.03	0.03	0.01	<0.01
Urine	5.10	12.6	14.4	25.6

[a]Values expressed as mean % derived from at least 4 dogs per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 10 mg/kg

[c]Not detectable

TABLE VI

PERCENT OF INJECTED DOSE RECOVERED IN MONKEYS[a]

TISSUES	HOURS AFTER DOSE[b]			
	2	4	8	24
Blood Serum	4.90	2.80	1.90	0.50
Spleen	0.09	0.02	0.05	*
Lungs	0.41	0.12	0.10	*
Heart	0.17	0.10	0.04	*
Liver	2.49	1.78	1.50	0.40
Kidneys	0.64	0.35	0.30	0.02
Intestine	2.86	1.51	1.30	0.90
Muscle	15.95	4.66	3.80	*
Brain	1.30	0.85	0.60	0.05
Bladder	0.13	0.04	0.04	< 0.01
Testes or Ovaries	0.02	0.01	< 0.01	< 0.01
Thyroid	< 0.01	< 0.01	< 0.01	< 0.01
Pancreas	0.10	0.09	0.05	0.02
Adrenals	< 0.01	< 0.01	< 0.01	< 0.01
Pituitary	< 0.01	< 0.01	< 0.01	*
Sp. Fluid	0.08	0.03	0.05	< 0.01
Urine	11.24	16.60	24.20	31.30

[a]Values expressed as mean % derived from at least 4 monkeys per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 10 mg/kg

*Not detectable

TABLE VII

PERCENT OF INJECTED DOSE RECOVERED IN RATS[a]

TISSUES	HOURS AFTER DOSE			
	2[b]	4[b]	8[c]	24[c]
Blood Serum	2.62	1.64	0.72	0.25
Spleen	0.07	*	*	*
Lungs	0.14	0.06	0.05	*
Heart	0.06	0.03	*	*
Liver	1.44	1.48	0.30	*
Kidneys	0.80	0.30	0.10	*
Intestine	0.77	0.62	0.54	0.14
Muscle	6.22	6.68	2.11	*
Brain	0.30	0.16	0.04	*
Bladder	0.04	0.02	0.01	*
Testes	----	0.31	0.12	*
Urine	12.60	20.45	18.24	39.86

[a]Values expressed as mean derived from at least 5 rats per group and calculated from ^{14}C radioactivity.

[b]Injected dose = 22 mg/kg

[c]Injected dose = 15 mg/kg

----Not performed

*Not detectable

TABLE VIII

PERCENT OF INJECTED DOSE RECOVERED IN MICE[a]

TISSUES	HOURS AFTER DOSE[b]		
	2	4	24
Blood Serum	0.41	0.60	0.40
Spleen	*	0.07	0.02
Lungs	*	0.07	0.08
Heart	*	0.04	0.06
Liver	0.94	1.59	1.10
Kidneys	0.05	0.47	0.32
Intestine	0.20	0.55	0.40
Muscle	*	2.48	1.55
Brain	0.04	0.16	0.06
Bladder	0.05	0.03	0.03
Testes	0.23	0.09	0.06
Urine	12.02	35.23	9.20

[a]Pooled values from 5 mice each

[b]Injected dose = 22 mg/kg

*Not detectable

TABLE IX

CONCENTRATION OF ^{14}C-MMH IN URINE
(ug/ml)

SPECIES	HOURS			
	2	4	8	24
DOG	341	802	507	109
MONKEY	885	341	290	233
RAT	170	1472	571	240
MOUSE	548	469	---	141

In terms of total excretion in the various species, the data in tables V thru VIII show the most rapid excretion by the mouse and, in decreasing order, by the rat, monkey, and dog, respectively. At 2 hours, the dog has excreted through the urinary tract approximately one-half as much as the monkey, rat, or mouse. No analysis of feces was attempted in this study; nor was there any attempt to measure respiratory $^{14}CO_2$ or other ^{14}C-labelled gases. A previous study on MMH was conducted in rats by Dost, Reed, and Wang at Oregon State University whereby they determined that 23 to 34% of the ^{14}C was expired in 27 hours, depending upon dose. With the exception of the mouse data, for which the authors have no ready explanation for the low figure at 24 hours, the total amount of radioactivity accounted for in the 24-hour studies ranged from 31% in the dog to 40% in the rat. Obviously, tissues such as bone, skin, fur, feces and perhaps excretion by the respiratory route, must account for the rest.

Table X shows the results of analysis of samples, both by chemical method and by measurement of radioactivity. These values are averages only and do not reflect the wide variability of individual results. The variability, however, does not change the fact that in nearly every instance in serum, and in every instance in urine, the radioactivity assay is higher than that measured by chemical means. By 24 hours, the pattern is clearly established that approximately 50% of that being excreted is unchanged monomethylhydrazine. The data indicate that each of the 4 species of test animals clears the material in a different way. This may be due either to difference in rate or metabolic pathway.

The most striking evidence of a difference between species is shown in table XI which is a summary of the toxic effects of monomethylhydrazine observed in dogs and monkeys. Emesis occurred in 16 out of 17 dogs tested and in only 5 of 16 monkeys. In the dog, the time to first appearance of emesis occurred most frequently at about 30 minutes after injection with MMH. In the monkey, where emesis occurred, the earliest time was at 95 minutes after injection, with one monkey becoming ill only after 7 hours had elapsed.

Convulsive seizures were not evident in any of the monkeys and occurred in almost all dogs. These animals received the same doses of MMH on a mg/kg basis and were treated in as nearly similar manner as was possible except for one major difference. The dogs were allowed spontaneous activity in a metabolic cage; the monkeys were immobilized in specially designed chairs. This immobilization and restraint may have resulted in the lesser incidence of convulsions in the monkeys. Convulsions in the dog occurred most frequently between 1 and 4 hours after injection, with the earliest time at 55 minutes and the latest onset time at 200 minutes. Seizures were nearly always multiple and

TABLE X

COMPARISON OF VALUES OBTAINED BY CHEMICAL ANALYSIS AND BY ^{14}C RADIOACTIVITY (ug/ml)

SPECIES	HOURS AFTER DOSE							
	2		4		8		24	
	CA	^{14}C	CA	^{14}C	CA	^{14}C	CA	^{14}C
Dog								
Serum	6.1	7.8	4.7	8.1	1.4	4.0	0.3	3.0
Urine	272	341	402	802	398	507	51	109
Monkey								
Serum	8.9	9.2	6.2	6.2	3.7	4.3	0.3	1.0
Urine	529	885	193	341	170	290	109	233
Rat								
Serum	4.4	8.0	5.9	11.6	0.3	5.0	0.3	0.7
Urine	128	170	1128	1472	74	571	91	240
Mouse								
Serum	1.2	2.9	2.0	4.2	0.3	*	ND	2.7
Urine	224	548	112	469	52	*	50	141

CA = chemical analysis

^{14}C = radioactivity expressed as MMH

*^{14}C data lost due to instrumental failure

ND = not detectable

TABLE XI

SUMMARY OF TOXIC EFFECTS OF ^{14}C-MONOMETHYLHYDRAZINE IN DOGS AND MONKEYS

SPECIES	NUMBER OF ANIMALS	DOSE mg/kg	TOXIC EFFECTS			
			EMESIS	CONVULSIONS	METHEMOGLOBINEMIA[1]	BLOODY URINE[2]
MONKEYS	16	10	5/16	0/16	0/16	0/16
DOGS	17	10	16/17	13/17	16/17	7/17

[1]DETERMINED AS GREATER THAN PRE-EXPOSURE LEVEL IN EITHER BLOOD OR URINE, OR BOTH.

[2]NO GROSSLY BLOODY URINES NOTED PRIOR TO 8 HOURS AFTER INJECTION.

successive, lasting approximately 3 minutes, and varying in severity from mild tremors to tonic-clonic convulsions. No convulsions occurred later than 7.5 hours after exposure.

Blood levels of methemoglobin were determined in both dogs and monkeys, both before and after exposure. Only in the dog was there an observed elevation of methemoglobin over preexposure levels. These levels, however, were not sufficiently high to account for the degree of toxicity elicited.

The most striking result noted in this study was the occurrence of gross hematuria in the dog and the obvious damage to the dog kidney upon gross examination at necropsy. This condition did not become grossly apparent until approximately 8 hours after injection, and all dogs held for 8 or 24 hours showed the same bloody urine. This occurred regardless of whether or not the animal had convulsed. In no instance was this phenomenon observed in monkeys or in rodents.

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UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, Ohio 45433

2a. REPORT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP
N/A

3. REPORT TITLE
DISTRIBUTION AND EXCRETION OF ^{14}C-MONOMETHYLHYDRAZINE

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Final Report, September 1965 - September 1966

5. AUTHOR(S) *(First name, middle initial, last name)*
Pinkerton, Mildred K.
Hagan, Edgar A.
Back, Kenneth C.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 1967	17	4

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 6302	AMRL-TR-67-175
c. Task No. 630202	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command, Wright-Patterson AFB, Ohio

13. ABSTRACT

A total of 20 mice, 20 rats, 17 dogs, and 16 monkeys received intraperitoneal injections of ^{14}C-monomethylhydrazine (MMH) at doses of 22 mg/kg (mice), 15 mg/kg (rats), and 10 mg/kg (monkeys and dogs). At 2, 4, 8, and 24 hours after exposure, representative samples of approximately 20 tissues from each animal were processed for ^{14}C assay using liquid scintillation counting techniques. Both blood and urine samples were simultaneously analyzed by a chemical colorimetric method for unchanged MMH, and the results were correlated with total ^{14}C content. Results of the ^{14}C assays indicated that the mouse, rat, and monkey excreted twice as much as the dog in the first 2 hours, and that all 4 species excreted 25-40% of the total dose by 24 hours after injection. Approximately 50% of the total ^{14}C excretion, at all experimental times, was apparently unchanged MMH as implied by the colorimetric results. Tissue distribution of ^{14}C showed the highest concentrations in liver, kidney, bladder, pancreas, and blood serum. Both clinically and pathologically, the dog was apparently much more susceptible than the other species tested to the toxic effects of MMH and to severe kidney damage.

DD FORM 1 NOV 65 1473

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Monomethylhydrazine Propellant Intoxication						